Letter to the Editor: Complete resonance assignments for the nudix hydrolase DR2356 of *Deinococcus radiodurans*

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1. Biological Context

Deinococcus radiodurans is a remarkably rugged organism, capable of withstanding high dosages of radiation as well as desiccation (Battista et al., 1999). A group of genes in the *D. radiodurans* genome code for enzymes that share a conserved sequence of amino acids dubbed the nudix box, for these enzymes all exhibit activity on nucleoside diphosphates linked to a moiety x (Bessman et al., 1996). It was initially thought that many of the *D. radiodurans* nudix proteins might be functional analogs of *E. coli* mutT, which hydrolyses mutagenic 8-oxo-GTP to prevent its frequent incorporation into DNA opposite adenine. (Fowler and Schaaper, 1997). It is now believed, however, that the nudix proteins play a role in the maintenance of homeostasis and physiological balance (Xu et al., 2001), though specific functions for the nudix proteins have not yet been determined.

All 21 of the nudix hydrolase proteins found in the *D. radiodurans* genome have been cloned and expressed, and spectrophotometric assays have been performed assessing activity on a range of substrates composed of nucleic acids and glyconucleic acids (Xu et al., 2001). Their study of the 144-residue DR2356 suggests that this protein hydrolyzes diadenosine tetraphosphate, which has been found in high concentrations in bacterial cells experiencing environmental stresses (Lee et al., 1983), such as a lack of nutrients or high exposure to heat or oxidation. In humans, Ap_4A has been found to have pharmicological effects on the cardiovascular and neurological system (Baxi and Vishwanatha, 1995). Though Ap_4A activity has been found in DR2356, it remains unclear exactly what role it plays in the overall endurance of *D. radiodurans* in the face of biologically extreme conditions. In this Letter we report the ^{1}H, ^{13}C, and ^{15}N resonance assignments for DR2356. Determining

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the NMR structure of this nudix hydrolase will facilitate studies of substrate binding and specificity and possibly provide insight to the nature of its catalytic activity.

2. Methods and experiments

A pET24a vector containing the gene for DR2356 was kindly provided by Michael Kennedy (Pacific Northwest National Laboratory) and transformed into *E. coli* Tuner cells (DE3). Doubly-labeled protein was expressed by growing and inducing cells in a minimal medium enriched with 1 g l^{-1} $(^{15}NH_4)_2SO_4$ and 2 g l^{-1} $^{13}C_6$-glucose for a total of 8 h. After washing, the cells were ruptured by sonication, and the protein was purified by ion-exchange chromatography over a DEAE Sepharose column (Pharmacia). The purified fraction was both concentrated and buffer-exchanged by centrifugation in Ultrafree-4 (Millipore) tubes. The NMR sample consisted of $\sim$ 1.3 mM protein, 10 mM Na_2HPO_4, 50 mM NaCl, 1 mM EDTA, 1 mM DTT, 0.05% NaN_3, 10% D_2O at pH 6.5. Experiments were conducted at 298 K on Bruker 600 MHz DRX and Bruker 500 MHz DRX spectrometers. Sequence specific resonance assignments were determined through data from the following 3D experiments: HNCA, HNCO, HNCACB, CBCA(CO)NH, HN(CA)CO, ^{15}N NOESY-HSQC, and HBHA(CO)NH. Sidechains were further assigned using an HCCH-TOCSY spectrum. Data were processed with NMRPipe (Delaglio et al., 1995) and analyzed and assigned with NMRDraw (Johnson and Blevins, 1994).

3. Extent of assignments and data deposition

Figure 1 shows the ^{1}H-^{15}N HSQC spectrum of ^{13}C-^{15}N uniformly labeled DR2356. Sequential assignments of backbone ^{15}N and amide protons were obtained for 130 residues, with those for residues 1-3 and 114 missing. C^α, C^β, and H^α were assigned for all residues 3-143 except 5. C' were assigned for all residues except 1-2, 70, 143 and 144. Most sidechain protons and carbons have been assigned.

Chemical shift assignments for DR2356 can be obtained with accession number 5602 at the BioMagResBank (www.bmrb.wisc.edu).

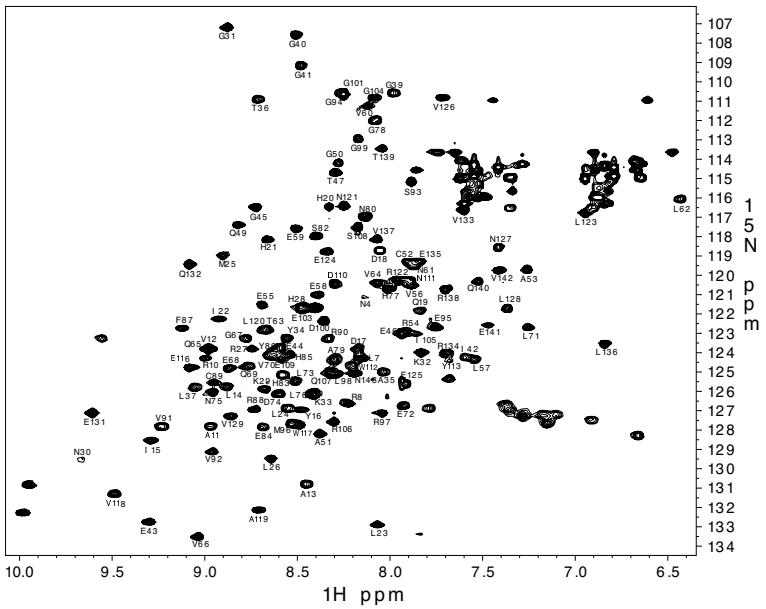

Figure 1. ^{1}H-^{15}N HSQC of DR2356 acquired at 298 K on a Bruker 500 MHz DRX spectrometer.

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